inhibitory effect of salicylamide on glucuronide and sulfate formation is most likely due to its very rapid metabolism. The nonlinear nature of salicylamide elimination prevents comparisons of formation-rate constants, but an admittedly approximate estimation based on available data (1, 8, 9) indicates that the formation of salicylamide glucuronide and sulfate proceeds about 10 times more rapidly than the formation of acetaminophen glucuronide and sulfate at body levels of 1 g. of these drugs. It is now evident that of the several combinations of acetaminophen, salicylate, and salicylamide studied in this laboratory, salicylamide is the major determinant in the biotransformation interactions encountered (1, 2).

REFERENCES

(1) G. Levy and H. Yamada, *J. Pharm. Sci.*, **60,** 215(1971).
(2) G. Levy and J. A. Procknal, *ibid.*, **57,** 1330(1968).
(3) E. J. Walaszek, *J. Amer. Med. Ass.*, **210,** 563(1969).
(4) M. W. Whitehouse and H. Boström, *Biochem. Pharmacol.*, **11,** 1175(1962).
(5) H. Boström, K. Berntsen, and M. W. Whitehouse, *ibid.*, **13,** 413(1964).
(6) R. M. Welch and A. H. Conney, *Clin. Chem.*, **11,** 1064(1965).
(7) G. Levy, *J. Pharm. Sci.*, **54,** 959(1965).
(8) G. Levy and T. Matsuzawa, *J. Pharmacol. Exp. Ther.*, **156,** 285(1967).
(9) W. H. Barr, *Drug Inform. Bull.*, **3,** 29(1969).

ACKNOWLEDGMENTS AND ADDRESSES

Received September 21, 1970, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication October 30, 1970.

Supported in part by Grant 5 R01 FD00015 from the National Institutes of Health.

Previous paper in this series: L. P. Amsel and G. Levy, *Proc. Soc. Exp. Biol. Med.*, **135,** 813(1970).

* Present address: A. B. Hässle, Goeteborg, Sweden.

MAO Activity in Normal, Cirrhotic, and Noncirrhotic Abnormal Human Liver

K. G. BHANSALI*, J. L. LACH, and J. A. CLIFTON

Abstract □ Normal, cirrhotic, and noncirrhotic abnormal human livers from autopsy and biopsy specimens were analyzed for MAO activity. The enzyme activity was expressed on the basis of liver weight, liver nitrogen, and mitochondrial nitrogen. When MAO activity was expressed on the basis of liver weight, the cirrhotic liver showed 80% less activity and noncirrhotic abnormal liver showed 35% less activity than normal liver. Liver nitrogen and mitochondrial nitrogen seem to be equally valid as reference substances for MAO activity.

Keyphrases □ MAO activity—human liver, normal, cirrhotic, noncirrhotic abnormal, comparison □ Enzyme activity—human liver, normal cirrhotic, noncirrhotic abnormal, comparison □ Liver, human, normal, cirrhotic, noncirrhotic abnormal—determination, comparison of MAO activity

It is difficult to classify diseases of the liver since pathological conditions affecting the liver may arise from cell degenerative process, circulatory disturbance, necrosis, and tumor formation. Liver diseases can be characterized, however, by careful histological study of the tissue. Cirrhosis of the liver is best characterized by distorted reconstruction of lobules throughout the entire liver or in a considerable part of it. One widely believed theory concerning cirrhosis states that the degeneration of the liver cells results from an accumulation of toxic amines in the organ to a level that exceeds the deamination ability of the metabolic processes (1). The accumulation of toxic amines may be due to the lack of an adequate MAO activity. The purpose of this investigation was to study the activity of this enzyme system in normal, cirrhotic, and noncirrhotic but abnormal human livers.

EXPERIMENTAL

Human liver specimens were obtained from autopsy as well as by biopsy from hospital patients[1]. Liver specimens were stored in the freezer as soon as they were obtained. The specimens preserved in cold showed reproducible results (2). The isolation technique of liver mitochondria was similar to that reported by Hogeboom *et al.* (3).

In this procedure, carried out in a cold atmosphere, an approximately 0.7-g. portion of fresh or frozen liver was chopped into small pieces with a dissecting scissor onto a watch glass and transferred to a tared glass homogenizing tube; the exact weight of the sample was recorded. Homogenization of this chopped tissue was carried out in 5 ml. of 0.88 *M* sucrose solution for 5 min. The homogenate was quantitatively transferred to a 50-ml. polyethylene tube. The homogenizing tube and pestle were thoroughly rinsed with three or four portions of 0.88 *M* sucrose solution, and the rinses were added to the polyethylene tube containing liver homogenate. The final total volume of homogenate, representing approximately 20 ml., was then centrifuged (cold room 0–5°) at $600\times g$ for 10 min., and the supernatant fraction containing mitochondria and microsomes was transferred by means of a pipet into a 50-ml. polyethylene tube. The supernatant containing mitochondria was centrifuged at $24{,}000\times g$ for 20 min. to sediment the mitochondria, and the supernatant portion containing microsomes was discarded.

The solid mass of mitochondria was resuspended in 20 ml. of 0.88 *M* sucrose solution and centrifuged at $24{,}000\times g$ for an additional 20 min. This supernatant fraction was discarded. The packed mass of mitochondria was quantitatively transferred into a homogenizing tube, and the suspension of mitochondria in phosphate buffer pH 6.9 (0.5 *M*) was facilitated by careful homogenization. The mitochondrial solution was then transferred into a 10-ml. volumetric flask, the homogenizing tube was carefully rinsed with phosphate buffer, and the volume was made to the mark with

[1] University of Iowa General Hospital and Veterans Administration Hospital of Iowa City, Iowa.

Table I—Normal Human Livers

Number	Subject	Sex[a]	Age	Liver[b] Specimen	mg. N_2/g. Liver	mg. Mitochondrial N_2/g. Liver	MAO Activity Expressed as: MAO Units[c]	MAO Units/Liver Nitrogen	MAO Units/Mitochondrial Nitrogen
1	R.D.	M	28 days	a	28.10	3.21	444	15.80	138.3
2	S.Mc.	F	2½ yr.	a	29.30	3.81	1381	47.15	362.5
3	D.C.	F	4 yr.	a	30.05	4.16	2170	35.34	255.2
4	S.V.	M	5½ yr.	a	39.75	5.16	2787	70.11	540.1
5	L.J.	F	8 yr.	a	28.46	2.91	972	34.15	340.2
6	J.W.	F	15 yr.	a	34.40	—	2649	77.00	—
7	L.H.	M	18 yr.	b	32.70	4.00	2715	83.02	678.2
8	C.H.	M	19 yr.	a	32.70	5.13	3150	96.21	614.0
9	B.S.	F	26 yr.	a	—	5.59	2880	—	515.2
10	T.B.	M	31 yr.	a	30.98	4.27	2640	85.21	618.3
11	A.T.	M	41 yr.	a	27.08	4.50	2520	93.05	560.0
12	M.G.	F	46 yr.	a	32.65	5.23	2580	80.59	493.9
13	M.B.	M	47 yr.	a	26.13	4.15	1968	74.80	478.2
14	M.K.	F	47 yr.	b	—	3.86	1892	—	490.2
15	C.J.	M	48 yr.	a	32.90	4.16	1924	59.40	462.5
16	O.M.	M	49 yr.	a	27.07	4.16	1087	40.15	261.3
17	R.D.	M	49 yr.	a	26.17	4.87	1358	51.89	280.0
18	W.P.	M	57 yr.	b	27.90	—	1852	66.37	—
19	S.R.	F	59 yr.	b	34.45	4.16	2805	81.42	674.3
20	R.S.	M	60 yr.	a	32.75	4.92	1845	52.33	375.0
21	C.W.	M	63 yr.	a	32.55	3.45	1380	42.39	400.0
22	S.M.	M	64 yr.	a	31.25	5.28	2327	74.43	440.5
23	W.F.	M	64 yr.	a	34.40	—	1800	52.32	—
24	B.H.	F	65 yr.	a	29.50	4.32	1897	64.37	439.6
25	C.B.	M	65 yr.	a	27.65	4.18	1253	45.31	299.8
26	E.W.	M	66 yr.	a	33.65	3.88	1713	50.90	441.5
27	A.Mc.	F	68 yr.	a	31.56	3.90	1704	53.99	436.9
28	L.B.	M	69 yr.	b	—	—	2565	—	—
29	W.T.	M	70 yr.	a	—	—	2527	—	—
30	C.T.	M	76 yr.	a	29.15	3.91	2037	69.87	520.9
31	C.D.	M	78 yr.	b	—	5.27	4493	—	852.6
32	E.Mc.	M	84 yr.	b	35.40	5.12	3070	86.72	599.6
33	M.T.	F	86 yr.	a	26.18	—	834	31.85	—
34	P.E.	M	94 yr.	a	27.15	4.17	1665	60.83	399.3
				Mean	30.78	4.37	2085	61.21	463.1
				SD	± 3.28	±0.70	± 785	±19.92	±144.0

[a] M = male, F = female. [b] a = autopsy, b = biopsy. [c] One unit is amount of MAO causing the disappearance of 1 mcg. tyramine/g. tissue/hr.

additional buffer solution. The enzyme activity of a 1.0-ml. aliquot of a mitochondrial solution was determined by the method of Sjoerdsma *et al.* (4), a method based on the colorimetric determination of substrate (tyramine) disappearance.

MAO activity was expressed on the basis of liver nitrogen as well as mitochondrial nitrogen. Nitrogen determination of liver was carried out by the semimicro Kjeldahl procedure using 100.0 mg. of sample. Nitrogen determination of mitochondrial solution was performed by the semimicro Kjeldahl procedure using 3.0 ml. of the aliquot solution. The results of the analysis are shown in Tables I–III. Statistical evaluation was by the Student *t* test.

RESULTS AND DISCUSSION

The data for 49 liver specimens are shown in Tables I–III, representing analysis of normal livers, cirrhotic livers, and noncirrhotic abnormal livers, respectively. It is difficult to state that a given specimen of biological material is normal. However, by common agreement, the histological appearance of biological material is used to establish normal, cirrhotic, and noncirrhotic abnormal categories. Livers showing hepatitis, fatty degeneration, and malignant changes were included in samples classified as noncirrhotic abnormal liver. Neither sex, age, nor mode of obtaining specimens (autopsy or biopsy) seemed to affect the results, so these parameters were ignored in evaluating the data.

Nitrogen content of normal, cirrhotic, and noncirrhotic abnormal human livers did not differ, and the values agree with the literature value of 27.3 mg./g. liver (5). However, data for the N_2 content of mitochondria of human liver have not been published. Various values of N_2 content of mitochondria of rat liver have been reported, depending upon the sedimentation technique. Liver mito-

Table II—Cirrhotic Human Livers

Number	Subject	Sex[a]	Age, yr.	Liver[b] Specimen	mg. N_2/g. Liver	mg. Mitochondrial N_2/g. Liver	MAO Activity Expressed as: MAO Units[c]	MAO Units/Liver Nitrogen	MAO Units/Mitochondrial Nitrogen
1	D.B.	F	9	b	—	—	135	—	—
2	D.V.	M	14	a	29.65	3.11	510	17.20	164.0
3	R.B.	F	26	b	29.44	—	536	18.20	—
4	E.O.	M	54	b	—	—	105	—	—
5	T.W.	M	59	a	22.85	—	203	10.72	—
6	E.H.	F	62	a	33.35	4.72	436	13.07	92.4
7	C.B.	M	62	a	28.09	1.98	934	33.26	477.2
				Mean	28.68	3.27	408	18.49	244.5
				SD	± 3.39	±0.35	±270	± 7.87	±160.0
							$p<0.001$	$p<0.001$	$p<0.05$

[a] M = male, F = female. [b] a = autopsy, b = biopsy. [c] One unit is amount of MAO causing the disappearance of 1 mcg. tyramine/g. tissue/hr.

Table III—Noncirrhotic Abnormal Human Livers

Number	Subject	Sex[a]	Age, yr.	Liver[b] Specimen	mg. N_2/ g. Liver	mg. Mitochondrial N_2/ g. Liver	MAO Activity Expressed as— MAO Units[c]	MAO Units/ Liver Nitrogen	MAO Units/ Mitochondrial Nitrogen
1	M.M.	F	11	a	29.56	4.56	1200	40.59	263.1
2	P.P.	F	31	a	21.19	3.40	1260	59.46	370.6
3	C.W.	M	32	a	31.70	4.79	2055	64.82	429.0
4	F.Z.	M	44	a	25.58	5.27	1449	56.64	274.9
5	J.D.	M	63	a	32.86	4.08	1875	57.06	495.5
6	L.C.	M	64	a	26.00	2.93	1155	44.42	394.2
7	G.W.	M	65	a	32.30	1.17	None[d]	None[d]	None[d]
8	P.W.	M	77	a	29.45	4.65	1320	44.82	283.9
				Mean	28.59	3.85	1473	52.54	358.9
				SD ±	3.77	±1.24	±326.3	± 8.48	± 81.6
							$p<0.10$	$p<0.30$	$p<0.10$

[a] M = male, F = female. [b] a = autopsy, b = biopsy. [c] One unit is amount of MAO causing the disappearance of 1 mcg. tyramine/g. tissue/hr. [d] Excluded in calculating the mean value.

chondria sedimented four times have been shown to have an N_2 value of 4.8 mg./g. liver (3), which also agrees well with the value found in this study. Unfortunately, the authors have values for mitochondrial N_2 in only three cirrhotic livers and cannot determine if the values differ from normal.

MAO activity of cirrhotic human liver is 80% less than that of normal liver if based on liver weight. That of noncirrhotic abnormal liver is 35% less than the normal liver when expressed similarly. These values further agree when MAO activity is expressed on the basis of liver N_2 as well as mitochondrial N_2.

Various enzyme activities such as esterase (7, 8) and oxidase (9) have been reported to be reduced in damaged hepatic tissue. In contrast, alkaline phosphatase activity has been shown higher in all types of hepatic injury (10). This study's data add one more example of a decrease in the liver enzyme activity in cirrhotic and noncirrhotic abnormal human livers. The deoxyribonucleic acid content of the same cirrhotic human liver has been reported to be 80% higher than the normal liver (11). The fall of hepatic MAO activity and rise in deoxyribonucleic acid content constitute a sensitive index of cirrhosis of human liver. Rats fed with pantothenic acid-deficient diet have also shown low MAO activity and high deoxyribonucleic acid content. However, those rats do not develop cirrhosis (12). Therefore, cirrhosis cannot be implicated as a cause of decreased MAO activity.

This study indicates 80 and 35% less MAO activity in cirrhotic liver and noncirrhotic abnormal liver, respectively, as compared to the normal liver. Nitrogen content of whole liver and mitochondria remain essentially constant in normal and pathological conditions.

REFERENCES

(1) "Liver, Structure and Function," H. Popper and F. Schaffner, Eds., McGraw-Hill, New York, N. Y., 1957, p. 523.

(2) K. G. Bhansali, Ph. D. thesis, University of Iowa, Iowa City, Iowa, 1960.

(3) G. H. Hogeboom, W. C. Schneider, and G. E. Pallade, *J. Biol. Chem.*, **172,** 619(1948).

(4) A. Sjoerdsma, T. E. Smith, T. D. Stevenson, and S. Udenfriend, *Proc. Soc. Exp. Biol. Med.*, **89,** 36(1955).

(5) E. M. Widdowson, R. A. McCance, and C. M. Spray, *Clin. Sci.*, **10,** 113(1951).

(6) S. A. Cucinell, A. H. Conney, M. Sansur, and J. J. Burns, *Clin. Pharmacol. Ther.*, **6,** 420(1965).

(7) S. Ellis, S. Sanders, and O. Bodansky, *J. Pharmacol. Exp. Ther.*, **91,** 255(1947).

(8) H. Popper, D. Koch-Weser, and J. J. de la Huerga, *Mt. Sinai Hosp.*, **19,** 256(1952).

(9) L. Benda, A. Locker, and E. Rissel, *Z. Ges. Exp. Med.*, **117,** 519(1951).

(10) R. A. Kritzler and J. Beubien, *Amer. J. Pathol.*, **25,** 1079 (1949).

(11) K. G. Bhansali, J. A. Clifton, and J. L. Lach, *J. Pharm. Sci.*, **58,** 1037(1969).

(12) *Ibid.*, **56,** 132(1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 11, 1970, from the *College of Pharmacy, University of Iowa, Iowa City, IA 52240*

Accepted for publication October 6, 1970.

Presented to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, Washington, D.C. meeting, April 1970.

This investigation was supported by Public Health Service Research Grant AM-0234-09 from the National Institutes of Health, Bethesda, Md.

The authors thank Dr. Edward Eugere, School of Pharmacy, Texas Southern University, Houston, Texas, for his criticism on the writing of this manuscript.

* Present address: School of Pharmacy, Texas Southern University, Houston, TX 77004